reflection-fluorescence correlation spectroscopy (ITIR-FCS) enabled direct determination of the local diffusion coefficients of CEACAM1 in different regions of the cell membrane while number and brightness analysis (N&B) and spatial intensity distribution analysis (SpIDA) provided insights into the oligomeric state of CEACAM1. These strategies for characterizing the dynamics of transmembrane receptors in live cells show clear promise for probing glycoprotein function in normal and disease states.

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Multimerization Studies of Membrane Receptors via μ-Patterned Surfaces

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Studying dimerization or multimerization processes of plasma-membrane localized receptor proteins is important for a better understanding of various cellular processes. While the physiological role of receptor multimerization remains a matter of debate for many proteins, it has been proved that such events are crucial for the function of different classes of membrane receptors (e.g. EGF or Insulin receptor). Several biochemical tools including cross-linking or co-IP assays are used to unravel protein multimerization. However, these techniques suffer from being indirect and hardly quantitative and give high number of false-positives and/or negatives. Indeed, there are biophysical assays to address this question, but the evaluation of the recorded data remains complicated and laborious. Here we describe how a combination of Total Internal Reflection Fluorescence (TIRF) microscopy and micro-structured surfaces (''micropatterning assay'') can be used to easily prove multimerization of membrane receptors. This technique was developed to detect protein-protein interactions (Schwarzenbacher et al., 2008; Weghuber et al., 2010) and offers the possibility to measure and quantify also weak or short-lived interactions in vivo. In a proof of concept experiment we studied the multimerization of YFP and/or CFP labeled ß1 and ß2 adrenergic receptors. The question of homo- and especially hetero-association of these G-protein coupled receptors (GPCR) is far from being completely understood. Receptor maturation, G-protein coupling, downstream signaling and regulatory processes such as internalization might be influenced by dimerized adrenergic receptors. We unequivocally show that ß1 as well as ß2 receptors form homo- and hetero multimers in living CHO-K1 cells. Our findings contribute to the field of adrenergic receptors. In general, our system might be of great interest for a fast and straightforward analysis of membrane-protein multimerization.

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Mobility of MenC and PhoU in Live E. Coli: A Single Molecule Tracking Study

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Innumerable signal transduction systems have evolved in which an environmental signal is transferred across the cell membrane to the cell interior via a series of protein movements and protein-protein interactions. Knowledge of the diffusion of signalling proteins in these networks and the effects of the presence of their interacting partners is required to understand the dynamics of the signalling response of such networks. Here, we will discuss our recent research using single molecule imaging techniques to determine the mobility of several proteins labelled by the fast folding variant of yellow fluorescent protein, Venus, in live E. coli. Specifically, we have determined the mobility of PhoU, a member of the PhoR-PhoB two-component signalling system and MenC, the o-succinylbenzoyl-CoA synthase, which has been found to interact with PhoU (Y.-J. Hsieh, Y. Yang, and B. L. Wanner, unpublished data). The effect of interactions of these proteins on their mobilities was investigated through monitoring protein diffusion in cells having deletions of the interacting partner genes. Results will be discussed in terms of their implications on the dynamics of signal transduction systems and in comparison to the known mobilities of other proteins and lipids in E. coli and eukaryotic cells.

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Cross Regulation in Signaling Networks: A FRET Study of the PhoB-PhoR Two-Component System in E-Coli

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The signal for the PhoR-PhoB two-component regulatory system of E. coli is environmental inorganic phosphate (Pi). A signal for environmental Pi insufficiency is propagated across the membrane to the cytosolic domain of the signalling histidine kinase PhoR via the ABC (ATP-binding cassette) transporter, the Pst (Pi-specific transport) system, in the absence of transport by protein-protein interactions, both in the membrane and the cytoplasm of the cell. Cross regulation also exists between PhoR-PhoB two-component system and key regulatory systems in E. coli, including the ArcB-ArcA two-component system for redox signalling. Such cross regulation among key signalling systems is thought to be important for integration of diverse cellular pathways in the control of cell growth and for survival under conditions of stress. Knowledge of how these interactions is brought about necessary to understand fully the dynamics of the response of the Pi signalling network to environmental Pi insufficiency and to understand interactions of the PhoR-PhoB and other signalling systems. Here we have employed Förster resonance energy transfer (FRET) techniques (acceptor photobleaching and sensitized emission) coupled with specific knock-out mutants to investigate in live E. coli cells interactions between ECFP-labelled members of the PhoR-PhoB system and protein targets with which they are thought to interact such as the o-succinylbenzoyl-CoA synthase, MenC. We will show a strong enhancement of the FRET signal through the use of protein fusions and knock-out mutants. We will discuss results of this study in terms of their implication on the dynamics of the PhoR-PhoB two-component signal transduction system.

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A Receptor Guanylyl Cyclase Reveals Auto-Phosphatase Activity

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In sea urchin sperm of Arbacia punctulata, a guanylyl cyclase (GC) serves as chemotaxis receptor that enables sperm to respond to a single molecule of the chemoattractant, resact. The efficiency of resact capture is high, because GC covers about 50% of the flagellar surface and binds resact with picomolar affinity. Furthermore, the binding affinity is controlled by the level of occupancy of the receptor. At high occupancy the resact affinity is lowered through negative cooperativity among subunits of the trimeric GC complex. The lifetime of active GC is controlled by its phosphorylation state. At rest GC is phosphorylated at six serine residues. After activation by resact, the GC becomes dephosphorylated with a biphasic time course, whereas dephosphorylation strongly coincides with the decrease of cGMP synthesis. During the initial fast phase the amplitude of GC dephosphorylation increases with the occupancy level. However, the time constant of this phase is independent of receptor occupancy. Moreover, dephosphorylation is superstoichiometric: even if only 5% of the GCs are occupied by resact, approximately 70% become dephosphorylated. We conclude from these results, that the occupied GC inactivates by auto-dephosporylation (fast phase) and additionally can dephosphorylate adjacent non-occupied GCs (slow phase). We could show for the first time that a receptor GC is regulated by auto-dephosphorylation.

Synaptic Transmission

3401-Pos Board B262

Conical Tomography of Ribbon Synapses of Light and Dark-Adapted Rod and Cones Photoreceptors

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Rod and cone photoreceptors undergo tonic transmitter release following changes in illumination. In both rods and cones, the fusion competent (i.e., docked and primed) synaptic vesicles are thought to be associated preferentially to a distinctive electron-dense structure referred as the ''ribbon.'' The aim of this study was to evaluate the location and number of docked (i.e., ''hemi-fused'') and fully fused (i.e., ''omega'' figures) vesicles in the axon terminal of rods and cones in light and dark-adapted mice. Using conical electron tomography, we reconstructed ribbon synapses of mice exposed to steady bright light or dark-adapted for 3 to 180 minutes. The conical tomograms were analyzed using density segmentation methods based on the watershed algorithm. We found that in the light, in both rods and cones the docked vesicular pool extended alongside the entire area of contact between the axon terminals and the horizontal cell endings. In rod axon terminals, the docked pool was comprised of 460-480 hemi-fused vesicles and remained essentially unchanged during dark-adaptation. In the dark, the terminals underwent rapid but reversible changes, including an increase in plasma membrane area and the number of ''omega'' figures (from ~190 to ~1300 in rods terminals). After ~30 minutes in the dark, the axon terminals returned to the conditions observed in mice exposed to steady light conditions. We thus conclude that in mouse rod and cone photoreceptors both ribbon-associated and non-ribbon regions of the